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Experimental Control of Damping-off

in Tomato Seedlings Transplanted from Sand, Including the Immediate Application of Fungicidal Drenches

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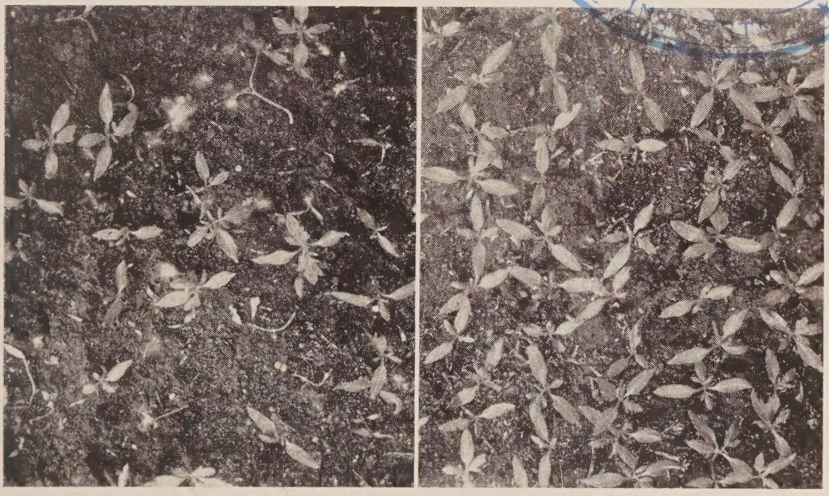

Control of damping-off in transplanted tomato seedlings by drying Pythium-infested soil. Left, infested and kept moist. Right, infested and thoroughly dried. Comparable control is possible by application of comparatively weak fungicidal drenches just before or after transplanting.

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Experimental Control of Damping-off

in Tomato Seedlings Transplanted from Sand, Including the Immediate Application of Fungicidal Drenches

W. S. Beach and Shu Yi Chen (Shuk Yee Chan) †

AS EARLY AS 1895, Atkinson *(2)* suggested that sand culture might aid in the control of damping-off of seedlings. In 1936, Dunlap *(4 and 5)* reported experiments showing the high degree of control which resulted from the use of a brown sand as a seed bed medium. This sand was washed in hot water at a temperature of 60° to 90° Centigrade and then supplied with a solution of plant nutrients that combined merely nitrogen, phosphorus, and potassium. Damping-off was prevented almost completely during germination and the post-emergence period.

By such sand culture, as many, or often more, healthy seedlings of various crops can be grown as in soil with the usual seed and soil treatments. Seedlings from sand are usually characterized by greater size, uniformity, and root development. The efficiency of sand in damping-off control appears to rest upon the fact that the washing in hot water removes not only the parasites but also the organic matter that promotes fungous growth and spread.

Soil treatment by steam, or by volatile disinfectants like formaldehyde, is recognized as an effective disease control measure, but it is regarded by many growers as too costly and cumbersome, a cause of delay in cultural procedures and not altogether free of difficulty in successful manipulation. Competition in the production of tomato plants for large commercial acreage leads growers to seek inexpensive as well as effective means to prevent loss by disease in their seed beds. In recent years there has arisen a disposition to rely solely on seed treatments followed by spraying or drenching the emerged seedlings with fungicides. Materials in use for treating tomato seed include mercury bi-chloride, organic mercury compounds, cuprous oxide, copper sulphate and hot water, or certain combinations of these. Cuprous oxide and organic mercury compounds are used as drenches or sprays after emergence. In this field, the research of Horsfall and his associates *(7 and 8)*

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upon copper sulphate and cuprous oxide and that of Wilson and Tilford *(12)* upon formaldehyde dusts has greatly improved the control of damping off in seed beds.

The severity of damping-off depends much upon moisture and temperature conditions that favor the parasites, yet only limited control is possible by regulation of these factors during adverse weather. Hemmi *(6)* showed that *Pythium debaryanum* and *Rhizoctonia solani* can be injurious over a wide range of temperature extending from 15° to 30° C. Alexander, Young and Kiger *(1)* found that *Pythium ultimum* is most active from 18° to 24° C. but can cause severe disease from 15° to 30° C.; that *Rhizoctonia solani* is most active at 24° C. but can be destructive much above or below this temperature. In respect to soil moisture these workers report that these fungi attack almost as severely when the water content is just sufficient for good growth (45 per cent of capacity) as when it is much greater. Their results show that only temperatures above 30° C. with water contents below 35 per cent arrest the development of the fungi.

The small amount of work and expense of sand culture together with its apparent advantage in damping-off control has led a number of tomato growers to adopt it to start their seedlings, particularly where stands from thickly sown seed are transplanted to flats of soil for wider spacing. While freedom from disease and well developed roots may enable seedlings to recover more quickly from the shock of transplanting, it is still possible for serious damping-off to occur within a few days when plants are set into infested soil. Such appears to be the case notably with seedlings started in sand. After a trial of sand culture, certain growers report that tomato seedlings grown in this way show unusual susceptibility to damping-off upon transplanting to soil. In order to ascertain whether there were factors in sand culture methods that result in abnormal susceptibility, and whether there were feasible measures for increasing resistance or otherwise overcoming this phase of damping-off, experimental work appeared necessary.

EXPERIMENTAL OBJECTS

Investigation was undertaken to determine: (a) if omission or insufficiency of magnesium, iron, sulphur, and the minor elements, or lack of balance in the nutrient solution increases susceptibility; (b) if the seedlings might be suitably hardened in preparation for transplanting not only by sunlight and restricted watering but also by using coarser type sand and raising the concentration of the nutrient solution through the addition of certain salts, particularly potassium sulphate; (c) if fungicides might be applied

to the soil nearer the time of transplanting than is usual, especially whether they might be included in the water normally supplied when plants are reset. This last phase of experiment was suggested by the fact that most of the damping-off occurs rather soon after transplanting. If an effective yet non-toxic dose of some fungicide could be applied to the soil before, during, or soon after transplanting, it would be more genuinely preventive than if applied after the development and functioning of new roots and the probable beginning of disease. Results pertaining to this phase of investigation have been reported in abstract *(3)*.

Since great variation in the percentages of damping-off is observed to occur under rather similar ranges of temperature and moisture, experiments were included to determine in what measure such variation might be caused by dampness of the soil and consequent vegetative activity of the parasites prior to transplanting.

MATERIALS AND METHODS

For a part of the experiments in nutrition, a white quartz sand excavated near Huntingdon, Pennsylvania, was used because the material was not likely to be a source of unknown available nutrients. It was fairly uniform in size of grains, the most of which were 0.2 to 0.5 millimeter in diameter. For comparison, a coarser, less uniform, brown alluvial sand from the Lycoming Valley, Pennsylvania, also was tested. The greater portion of the grains in this sand were 0.4 to 0.8 mm in diameter, but there were also considerable numbers of smaller and larger particles. The water holding capacities of these sands were respectively 24 and 15 per cent of the dry weights, the former being the same as the sea sand used by Dunlap *(4)*. When each kind of sand was wet to full capacity, the rates of water loss by evaporation differed little for the first 3 days, but at the end of 6 days the quartz sand retained 6 per cent and the alluvial 1.2 per cent of the dry weight. The difference in absorption of radiant heat was shown by the alluvial becoming as much as 3.5° C. warmer than the quartz in winter sunlight in the greenhouse.

The sands were washed in small quantities for 15 minutes with a hose extended from a hot water tap which maintained a temperature of 62° C. Fine particles of soil and organic matter were removed until no cloudiness appeared when the sands were agitated with water. They were then transferred to new or disinfected flats and dried until scant moisture remained.

In dissolving and applying nutrient materials to the sands, water was used at the rate of a liter per flat, or about a quart to the square foot. This gave the quartz sand approximately half of its water

holding capacity, and subsequent watering was done so as to maintain nearly this same degree of moisture. Only a single application of nutrients was made, and this just after seeding. As checks, seedlings were started in both untreated and pasteurized soils. All tomato seed used had been treated by the grower with Semesan, and this was done by soaking seed for 5 minutes in a 1 to 1200 solution and then drying.

The soil for transplanting was the usual mixture of one-half loam, one-fourth sand, and one-fourth composted manure. When preliminary results indicated that this mixture did not harbor or promote much disease, uniform infestation was undertaken using pure cultures of either *Pythium ultimum* Trow. or *Rhizoctonia solani* Kühn from tomato. In the case of Pythium, two different forms of inoculum were used: one grown on cornmeal agar in which plentiful conidia and oospores occurred, the other grown on steamed rye in which the growth was mostly mycelium. In preparing the steamed rye medium, two parts of whole grain were used with three parts of water. The inoculum on cornmeal agar gave more uniformity but little increase of disease, while that on steamed rye gave much increase but no great uniformity. With either form, it was found important to place the inoculum near the surface of the soil. The Rhizoctonia inoculum consisted of cultures on steamed rye; these were dried, coarsely ground, and then mixed with the soil. A high percentage of disease generally was produced.

The seedlings were transplanted when the first true leaves were beginning to develop; from the standpoint of prompt recovery no advantage was found in greater age. Experiments showed that the seedlings transplanted one week after emergence were more advanced in growth than those transplanted two weeks after emergence if comparison was made a month following the date of sowing. One test showed 46 per cent greater weight of random samples in favor of the former period.

The unit test consisted of 100 seedlings transplanted to a flat having 3 by 10 by 6 inches inside measurements. As a rule, four replications were made. Aside from the data summarized in Table 1, the plants in all treatments for direct comparison were transplanted the same day and kept under like conditions of light, temperature, and humidity as far as practicable. Conditions favoring damping-off were provided by: (a) keeping the soil comparatively wet for a week, (b) placing the flats for 4 of 5 days in a moist chamber covered with either muslin or glass, and (c) maintaining the temperature as near as possible to 24° C. Although temperatures varied somewhat, they were within the range suitable for active growth of the damping-off fungi.

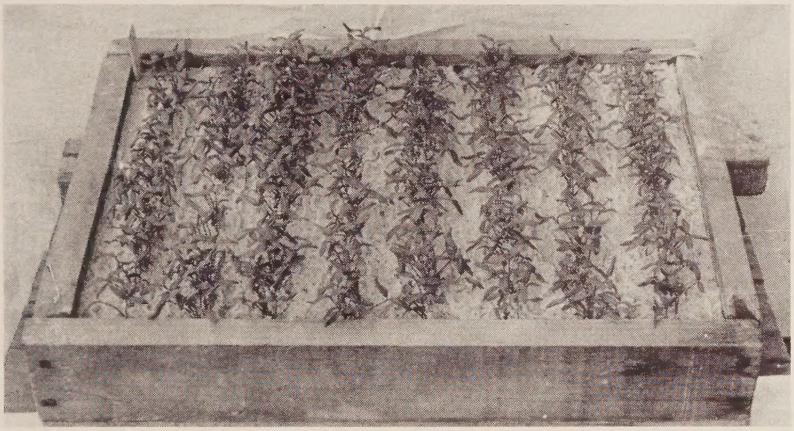

Fig. 1.—Tomato seedlings in quartz sand showing the stage of growth at transplanting.

Data upon the number of surviving seedlings were taken 10 days after transplanting, although most of the damping-off occurred during the second or third days. The death of a plant was in general the criterion of disease but cases were included in which stems fell prone or broke readily from the roots. When fungicides had been applied, random samples of 25 plants were removed at the time survival counts were made, or later according to season, and weighings were made to ascertain better the effects, upon growth.

CAUSAL ORGANISMS

In the present investigation, various isolations from damped-off tomato seedlings from untreated soil indicated that *Pythium ultimum* Trow. was the cause of the disease in all but a small proportion of cases. This agrees with the report of Horsfall from New York *(7)* as well as that of Alexander, Young and Kiger *(1)* from Ohio. In one collection of diseased tomato seedlings taken from unwashed sand being used a second time without disinfection in a commercial greenhouse, *Rhizoctonia solani* Kühn was common. Pythium also was found under similar conditions in sand. Such sand containing the dead plants was used for infestation in certain experiments.

In an examination of the washed roots and stems of 3200 tomato seedlings from untreated soil, discernible brown lesions were found on 12.7 per cent, although less than ½ per cent actually damped-off, for favorable conditions for damping-off had not been maintained. Isolations from a random lot of specimens with lesions yielded

Pythium in 83 per cent of cases. Discernible lesions of any character on the experimental seedlings from sand culture were less than 1 per cent, and these seldom yielded damping-off fungi.

EFFECTS OF SEED FLAT TREATMENTS UPON DAMPING-OFF AFTER TRANSPLANTING

To determine whether nutrition in the seed flats of sand could influence susceptibility to damping-off after transplanting, or otherwise affect the ability of the seedlings to withstand this operation, the following different materials were tested:

1. THE NUTRIENTS COMMONLY RECOMMENDED TO GROWERS. For the quantity of sand held by flats of 3 by 10 by 6 inches inside measurement, the proportions were 5 grams of 20 per cent commercial superphosphate and 3 grams of saltpetre (potassium nitrate) per liter of solution, or per flat. These materials were fixed with water and the solution afterwards decanted to remove insoluble matter. For brevity, this solution is referred to as the superphosphate-saltpetre. It may be stated that the preceding proportions are equivalent to approximately 2 ounces of superphosphate and 1 ounce of saltpetre to the square yard with the sand 4 to 5 inches deep as in the case of most commercial beds. If pure calcium acid phosphate is used instead of superphosphate, 0.4 ounce to a square yard is a suitable proportion.

2. A DILUTE UNBALANCED SOLUTION consisting of 1 gram of potassium nitrate and 0.2 gram of calcium acid phosphate per liter.

3. A MODIFIED KNOPS'S SOLUTION consisting of 1 gram of calcium nitrate, 0.25 gram of potassium acid phosphate, 0.25 gram of magnesium sulphate, 0.25 gram of potassium chloride, 0.01 gram of iron phosphate and also traces of the salts of manganese, zinc, boron, and copper per liter.

4. A RELATIVELY CONCENTRATED SOLUTION containing 3 grams of calcium nitrate, 1.5 grams of potassium acid phosphate, and 1 gram of magnesium sulphate per liter. This is designated as the concentrated 3-salt solution. Nightingale and Farnham *(11)* in their study of the anatomy, metabolism, and bud abscission of the sweet pea report an increase of hard tissue development with the use of the higher concentrations of these salts. They compared concentrations of 0.5, 1, 2, and 3 atmospheres.

5. THE UNTREATED SOIL MIXTURE as a check.

6. THE SOIL MIXTURE PASTEURIZED BY STEAM as a check.

Apparently good seedlings were produced by all the nutrient solutions in combination with either kind of sand. There were no marked distinctions in size and vigor among the seedlings in a single kind of sand as the first true leaves were developing. Seed-

lings in alluvial sand sometimes exhibited both a deeper green and purple coloration than those in quartz. Seedlings in soil exhibited greater depth of color than those in either kind of sand. There were differences among various seed flat treatments in respect to the length of time between sowing of seed and the emergence of seedlings. A typical example is the following sequence of emergence in which four days elapsed between the first and the last: (a) alluvial sand, (b) steamed soil, (c) untreated soil, (d) quartz sand, and (e) formaldehyde treated soil. As a rule, six to eight days elapsed between full emergence and transplanting.

Under the moist chamber conditions which were provided to promote fungous growth and infection, the transplanted seedlings were observed to pass through a period of great susceptibility to Pythium. This lasted approximately three days as shown by counts of loss. This occurred irrespective of the particular seed flat treatment and is illustrated by relative losses at the end of the third and tenth days. In some seedlings from steamed soil, the final losses in 4 different transplantings after 10 days were 54, 23, 25 and 14 per cent, but at the end of 3 days they had been 49, 22, 19 and 12 per cent. As a rule, signs of damping-off were discernible early the second day, and susceptibility ended seemingly as soon as new roots were functioning and the plants could maintain normal turgor. With *Rhizoctonia solani,* however, the period of attack was much longer or indefinite, and various degrees of injury were possible.

The average losses by damping-off suffered by seedlings from the differently treated seed flats in all series of transplanting to steamed soil and to soil infested with oospores and conidia on agar are summarized in table 1 with the results from the two kinds of sand combined. The average losses were quite similar for seedlings from sand supplied with the superphosphate-saltpetre and Knop's solutions and from steamed soil when transplanted to infested soil; the percentages were respectively 5.63, 6.88, and 6.6. These losses were less than half of that for seedlings started in untreated soil for which the per cent was 14.2.

Seedlings started with the dilute unbalanced solution often sustained losses considerably greater than seedlings started with the other solutions, the average being 12.8 per cent. Results for the concentrated 3-salt solution were rather similar to those of the superphosphate-saltpetre and Knop's except for one series in which it produced slight chemical injury. Its average was 10.9 per cent. The consistently wide difference between steamed and infested soil used for transplanting afford proof that the plants were destroyed chiefly by fungous attack.

Table 1.—Average percentages of damping-off suffered by seedlings from differently treated seed flats when transplanted to Pythium-infested* and steamed soil; 100 seedlings in each replication.

SEED FLAT SOLUTION IN SAND, OR SOIL TREATMENT	TRANSPLANTED TO INFESTED SOIL		TRANSPLANTED TO STEAMED SOIL	
	Replications	Damping-off	Replications	Damping off
		per cent		*per cent*
Superphosphate-saltpetre	27	5.63	12	0.24
Dilute unbalanced	28	12.8	14	3.88
Knop's	35	6.88	16	1.41
Concentrated 3-salt	24	10.9	12	0.33
Steamed soil	16	6.9	8	0.16
Untreated soil	18	14.2	12	2.82

* Pythium inoculum grown on cornmeal agar.

The significance of the observed differences in the percentages of damping-off resulting from the various seed flat treatments could be ascertained only by treating separately the data of each series of transplanting by analysis of variance. This was not only a consequence of changes in such environmental factors as light, temperature, and moisture; the results of soil infestation were found to vary considerably among successive series.

There were four series in which steamed soil, Knop's, the superphosphate-saltpetre, and the concentrated 3-salt solution were compared. In no series was there a significant difference in damping-off among seedlings from steamed soil, the superphosphate-saltpetre, and Knop's solution, while the concentrated 3-salt solution was inferior to the three other materials only in the single series in which it gave chemical injury. In four out of six series, the dilute unbalanced solution was inferior to the superphosphate-saltpetre and Knop's, yet it was usually better than untreated soil.

There proved to be no significant difference in the effects of the two kinds of sand when transplanting was simultaneous and environmental conditions were alike, although an apparent trend toward lower losses on the part of the alluvial was noted in certain trials. In transplanting from sand or steamed soil to steamed soil, losses were usually less than 1 per cent. There was only one series in which losses were great enough to indicate recontamination of the pasteurized soil and this occurred with seedlings from the dilute unbalanced and Knop's solution. For this reason the average losses for these solutions were higher than the others, being respectively 3.88 and 1.41 per cent, table 1.

In transplanting seedlings from untreated soil to steamed soil, the average loss of 2.82 per cent may not seem important, yet it was relatively great in comparison with the 0.16 per cent loss in transplanting from steamed soil to steamed soil, the ratio being 17.6 to 1. In these particular transplantings, conditions did not promote a great deal of disease. Although the corresponding losses of 14.2 and 6.6 per cent in transplanting to infested soil were actually larger, they have a ratio of only 2.15 to 1. These ratios indicate the value of disease-free seedlings over those that carry some infection.

Experiments with Mycelial Infestation

Experimentally, an incidence of infection by Pythium as high as 50 per cent was obtained with assurance only by artificial infestation with the inoculum grown on steamed rye. This was placed in the soil a half inch below the surface by opening furrows in line with the rows of seedlings to be set later. The kernels bearing fresh mycelium were deposited at quarter-inch intervals and covered with soil. This method of infestation was used to make further comparison of seedlings from steamed soil and alluvial sand supplied with the superphosphate-saltpetre nutrients. The seedlings from steamed soil sustained an average loss of 47.5 per cent while those from the sand an average loss of 53 per cent. The difference was not significant. The suspected difference in susceptibility between seedlings from quartz and alluvial sand was tested by the same method of mycelial infestation, the seedlings being started with Knop's solution. Those from quartz sand sustained an average loss of 46.5 per cent and those from alluvial 48 per cent. The difference was not significant.

Effects of Excess Potassium Sulphate and Reduced Water Supply

The experiments to harden seedlings were limited to the short period between complete emergence and transplanting. As this period was rarely more than six to eight days, the possibility of hard tissue development and gain of resistance was restricted in respect to time.

In the experiments with excess potassium sulphate, this salt was added in varying quantities to quartz sand supplied with the superphosphate-saltpetre nutrients. No theoretical basis is presented here for the use of the excess potassium sulphate except to create a xerophytic root environment and possibly increase the development of sclerenchyma tissues as indicated by Nightingale and Farnham *(11)*.

Tomato seedlings were found tolerant to a considerable excess of potassium sulphate; it was possible to add 5 grams to a square

foot at sowing without apparent interference with germination, provided the sand was kept well supplied with moisture. By gradual increase after emergence, it was possible to reach 9 grams to the square foot with satisfactory growth if there was no great decline in moisture. With increase to 12 grams, root injury and serious growth retardation could not be avoided. From seed flats in which the excess had been built up to 9 grams, 600 seedlings were transplanted to Pythium infested soil. These seedlings sustained a loss of 9.5 per cent in contrast with 12.8 per cent in the same number of checks. Analysis of variance showed this difference to be significant at 19 to 1 odds.

Experiments were conducted in which sand was supplied with the superphosphate-saltpetre nutrients in double proportion and the tomato seedlings were grown to the seed leaf stage. The sand was then permitted to dry during a few days when the temperature was 90° to 100° Fahrenheit. Wilting and evidence of chemical injury on the finer roots occurred. In the absence of damping-off fungi, the seedlings showed great capacity for recovery when an ample supply of moisture and moderate temperature was maintained again. The development of new rootlets enabled seedlings to be transplanted with fair success.

Two series of experiments with heavy and light watering were conducted during winter with the use of quartz sand and the superphosphate-saltpetre nutrients. After full emergence of the seedlings, one set of flats was kept near the water holding capacity of the sand while another set was given no more water but narrow furrows were made between the rows to hasten drying so that the moisture content of the sand fell to about 6 per cent of the dry weight. Altogether 1100 seedlings (11 replications) were transplanted from each of the respective treatments to Pythium infested soil. The high moisture treatment resulted in an average of 10.9 per cent damping-off and the low moisture 10.07 per cent, indicating no difference in resistance.

A series of experiments was conducted which involved moisture limitation in respect to both the seedlings and the parasite. Seedlings were started in steamed soil, and after full emergence and growth for one week, watering was restricted so that there were slight signs of wilting at midday. Transplanting was delayed until the plants were 16 days old and the first true leaves were well developed. A part of these seedlings were set in Pythium infested soil that had become thoroughly dry over summer, another part in soil with recent oospore and conidia infestation, and the rest in soil with recent infestation with mycelium on steamed rye. In the first case, the four replications continued a perfect stand, in

the second the average loss was 9.8 per cent and in the last 29 per cent. The results do not indicate that the seedlings were hardened enough to resist markedly attack by active vegetative mycelium.

Effects of Drying upon Pythium Infested Soil and Pythium Inoculum

In the preceding paragraph, wide differences in loss ranging from none to 29 per cent have been reported among (a) an infested soil that has become dry, (b) a soil containing fresh spores and conidia, and (c) a soil harboring actively growing mycelium. A further study of drying upon Pythium was made by drying for 10 days flats of soil in which seedlings had suffered losses approximating 50 per cent. The soil was then pulverized and set again with tomato seedlings. Perfect stands were a common result despite the fact that the flats were held in the moist chamber five days with the soil comparatively wet. This inhibiting effect of drying upon damping-off was noted several times in the present work since check flats usually underwent drying and pulverizing before reuse. Incidence of disease was low without a renewal of infestation by pure cultures.

In the drying of the soil, the fungus either perished or there remained only spores which could not germinate and form mycelium before the seedlings passed their period of high susceptibility. Drying and aging reduced rapidly the potency of the Pythium inoculum on steamed rye. Some that had produced approximately 50 per cent loss when used fresh for soil infestation caused only 23 per cent loss after drying 24 hours in an open dish. When flats infested with inoculum dried in this way were set aside on the open bench for 24 hours before transplanting, damping-off fell to 4 per cent. These results serve to illustrate how much variation in experimental procedure could vary incidence of disease.

APPLYING FUNGICIDES TO THE TRANSPLANTING SOIL

The experiments with fungicides involved ranges of dosage applied as closely as practicable to the time of transplanting. The primary object was to find a safe and effective amount of a material that would provide protection during the most critical period of fungous attack, which, as mentioned, embraces about three days following the change to infested soil. The fungicides were applied to prepared flats of soil not more than a day before or immediately after transplanting. A drench of a pint of water to the square foot of surface (0.5 liters on flats of 160 square inches) was used to apply all soluble or suspendable substances irrespective of concentration.

When drenches were used before transplanting, it was necessary to set the flats aside for six to eight hours until the dampened

soil became workable, and after the seedlings were set, they were given additional water as required. When drenches were used after transplanting, the pint of water containing the fungicide provided insufficient moisture for the plants; and so more was sprinkled upon them, and this served to rinse injurious chemicals from the foliage. Most of the results for determination of the best dosage, which are recorded in the accompanying tables, involve application before transplanting, but when it appeared that treatment afterwards would entail less delay in cultural procedure, tests of this kind were undertaken.

Although tests were conducted with the following fungicides: cuprous oxide, bordeaux, mercurous chloride (calomel), mercuric chloride, zinc chloride, certain organic mercury compounds, tetrachloro-para-benzoquinone (Spergon) and copper sulphate, this paper reports only the results given by copper sulphate, low amounts of hydroxymercurichlorophenol (Semesan) and tetrachloro-para-benzoquinone. With these, effective control without significant injury was obtained. The seedlings for these experiments were started in sand using mostly the superphosphate-saltpetre nutrients, but Knop's and the concentrated 3-salt solutions were sometimes used.

Copper Sulphate Drenches

The fungicidal properties of copper sulphate are well known, particularly against species of Pythium and Phytophthora in soil. Newhall and Chupp *(10)* discuss the application of copper sulphate solution to ground seed beds prior to sowing, mentioning a pound of bluestone dissolved in 25 gallons of water as sufficient for 100 square feet of surface. Such use of the fungicide has been limited on account of the risk of injury to many crops, or because their tolerance had to be determined definitely for each particular condition. Tomatoes have great tolerance for copper sulphate, but Horsfall *(7)* found that seedlings are definitely injured if transplanted into soil treated with a 0.5 per cent solution at the rate of a liter per square foot. This amount is equivalent to 5 grams to the square foot, while 1 pound to 100 square feet is but slightly less, or 4.54 grams to the square foot. These proportions exceed greatly any tried in the current experiments. Details of several series of tests with this chemical are reported since they indicate what amounts are stimulative as well as fungicidal and safe.

The beneficial effects of small amounts of copper sulphate on transplanted tomato seedlings were impressive in the first series. This consisted of seven flats each treated with 0.4 gram (0.36 gram

to the square foot[1]) and seven untreated. The average stand of the treated flats was 99.4 per cent while that of the untreated was 87.3 per cent, and every treated flat was better than any untreated. There was marked stimulation of growth and this was likewise wholly consistent. The average weight of random samples of 25 plants 10 days after transplanting from treated flats was 26.2 grams in contrast with 16.5 grams from untreated. These results were obtained during March when many seedlings are started.

The next series of tests was during April and included six different rates of application ranging from 0.1 to 1 gram per flat with treatments in duplicate. Results were irregular. The highest application of 1 gram gave effective control with an average stand of 98.5 per cent while the low amounts of 0.1 and 0.167 gram gave partial control. The intermediate amounts of 0.25 and 0.5 gram produced no reduction of loss, or results were within the range of the checks, the average stand of which was 86.7 per cent. The cause of this irregularity may not be fully understood, but it appeared due to the fact that some of the flats were transplanted in the forenoon, mainly those receiving the intermediate treatments, and they were unduly exposed to direct sunlight when the temperature ranged from 25° to 30° C. The tendency to wilt was greater than usual, and watering in addition to that given when the seedlings were set was provided during the day. It is possible that much of the copper sulphate was leached away while conditions greatly increased susceptibility. Consideration may be given to the possibility of chemical injury when the foliage was wet and the temperature was high, although the success of the strongest treatment detracts from this.

Since the previous experiments had revealed no definite relation between the concentration of copper sulphate and the degree of control, another series was run during May. The experiments were planned so that the data could be treated by an analysis of variance, each rate of application having four replications. A strong concentration of 1.8 grams to the square foot was included to see whether such an amount would prove toxic. A surplus of flats made it possible to have eight checks. The season was favorable to rapid growth and the incidence of disease in the checks was less than the general average. The data for the percentage of stand and the weights of random samples are given in table 2.

All rates of application in table 2 show significant reductions of damping-off in comparison with either set of checks as a difference of only 2.89 per cent is required for significance at 99 to 1 odds and

[1] In planning the experiments, the dosage was based on the flats of 160 square inches soil area with applications varying by tenths of gram. In converting this rate of dosage to the square foot basis, each amount was reduced by 10 per cent.

Table 2.—Control of damping-off and increased growth of tomato seedlings resulting from various rates of copper sulphate application on flats of soil infested with *Pythium ultimum*; tests conducted from May 20 to June 10.

AMOUNT OF COPPER SULPHATE	AVERAGE STAND OF SURVIVING PLANTS IN 4 REPLICATIONS	AVERAGE WEIGHT OF RANDOM SAMPLES OF 25 PLANTS 10 DAYS AFTER TRANSPLANTING
gm. per sq. ft.	*per cent*	*grams*
0.113	99.25	30.9
0.15	96.25	30
0.225	97.25	26.3
0.45	99	28.12
0.9	99.25	27.45
1.8	100	22.2
0	93.75	22.3
0	92.75	22.75

Difference between averages of stand required for significance at 19 to 1 odds, 2.03 percent; at 99 to 1 odds, 2.89 per cent.

Difference between averages of weight required for significance at 19 to 1 odds, 4.48 grams; at 99 to 1 odds, 6.36 grams.

the averages for all rates of treatment exceed the checks in greater degree. Significant growth stimulation is exhibited by 4 rates of treatment at 19 to 1 odds, but only by the 2 lowest at 99 to 1 odds. The rate of 1.8 grams appears to be within a range of dosage that is not stimulative. This series failed to show any correlation between the strength of the copper sulphate and the degree of disease control or plant survival. This feature of the results suggests a question whether the increases of stand may not be due more to growth stimulation than to fungicidal action.

With an endeavor to obtain a higher incidence of damping-off in the checks, further series of experiments with copper sulphate were conducted during the subsequent fall months. In one series, the soil was infested with *Pythium ultimum* on steamed rye but the cultures were partially dried, coarsely pulverized, and then mixed uniformly with the soil. These cultures showed little sporulation in comparison with those on cornmeal agar but they were easier to break into fragments to gain distribution. As special checks, flats of naturally infested soil were transplanted without any treatment. The results of this series are given in table 3 with the percentages of stand of individual replications included to illustrate the range of variation.

The incidence of damping-off in the checks was only slightly greater than the general average. While the naturally infested soil exhibited 6 per cent smaller loss than that receiving the in-

Table 3.—Control of damping-off in tomato seedlings by copper sulphate drenches applied the day before transplanting to flats of soil infested with *Pythium ultimum;* tests conducted in November.

Amount of Copper Sulphate	Stand of Surviving Seedlings in 100-plant Tests				
	Replications				
	1	2	3	4	Average
gm. per sq. ft.					
0.9	99	98	99	100	99
0.6	99	98	100	96	98.25
0.45	98	93	90	94	93.75
0.3	94	100	97	97	97
0.225	90	96	95	91	93
0	88	87	74	79	82
0 *	96	89	76	91	88

* Naturally infested soil.

Difference between averages of treatments required for significance: at 19 to 1 odds, 7.9; at 99 to 1 odds, 9.75.

oculum on steamed rye, analysis of variance does not show this to be significant. Apparently the drying had been sufficient to destroy largely the active mycelium. All the treatments ranging from 0.9 to 0.225 gram were significantly better than the artificially infested checks but again no significant difference appeared among the various rates of copper sulphate application.

When flats of soil were infested with fresh Pythium mycelium on steamed rye or with either fresh or dried Rhizoctonia inoculum, copper sulphate applications ranging from 0.5 to 1 gram to the flat hardly gave a satisfactory degree of control. When losses were as great as 50 per cent in the checks, treated flats suffered losses from 15 to 20 per cent. The disease conditions set up in these experiments, however, can be regarded as exceptionally severe and such as can be much alleviated if not avoided by cultural practices mentioned in this paper.

In tests of copper sulphate added to the nutrients in flats of sand, seedlings began to exhibit appreciable growth retardation and root injury at a concentration of 1:6000. This is equivalent to 0.166 gram to the square foot on the basis proportions tested in soil. Evidently materials in soil adsorb or react with the chemical so that much greater amounts can be added with safety to soil than to sand. It is probable that a similar relationship prevails in the use of various nutrient salts.

When copper sulphate was added to potato dextrose agar, proportions ranging from 1:2000 to 1:12000 completely inhibited the growth of young Pythium mycelium transferred to the surface of this medium in petri dishes; but when infected stems of tomato

seedlings were transferred instead, gnarled and abnormal mycelium of the fungus was able to grow a few millimeters in apparent contact with this fungicide containing medium. In the presence of much organic matter in the soil, the comparatively small amounts of chemical probably inhibit rather than destroy the parasite growing out from old host tissues.

Semesan Drenches Applied Before or Immediately After Transplanting

The amount of Semesan (hydroxymercurichlorophenol) which the manufacturer specifies for drenching seedbeds to prevent damping-off after the seedlings have emerged is approximately 1.2 grams to the square foot. This is based on the recommendation of an ounce of this fungicide in 3 gallons of water spread at the usual rate of a pint to the square foot. Preliminary tests in the present research indicated that considerably smaller amounts would give effective control of the damping-off accompanying transplanting although Rhizoctonia was more or less a factor, and the danger of toxic effects would be mostly avoided. Furthermore, the usual stronger application might be used after the seedlings had formed new roots if a second treatment appeared necessary.

The results of formal experiments with varying small amounts of Semesan applied immediately after transplanting are recorded in table 4. The soil was infested with cultures of *Rhizoctonia solani* on steamed rye which were dried and pulverized before use. The drenches were followed by a light rinsing with water.

According to analysis of variance, treatments of 0.9, 0.675, 0.45 and 0.225 gram to the square foot all give equivalent results as the differences between the respective averages of control are all considerably less than 9.27 per cent which is required for significance at 19 to 1 odds. As shown by the untreated replications, the attack of the fungus was severe under the moist chamber conditions for

Table 4.—Control of damping-off and effects upon growth of tomato seedlings resulting from application of Semesan drenches immediately after transplanting to flats of soil infested with *Rhizoctonia solani* from tomato; tests conducted in late October.

Amount of Semesan	Number of 100-Seedling Replications	Average Surviving Plants*	Average Weight of Random Samples of 25 Plants
gm. per sq. ft.		*per cent*	*grams*
0.9	5	93	22.1
0.675	5	95.4	21.7
0.45	5	94.6	25.05
0.225	5	91.4	25.6
0	5	58.8	17.92

* Difference between averages of treatments required for significance in the case of surviving plants at 19 to 1 odds is 9.27 per cent.

the average of surviving plants is only 58.8 per cent. The treated plants show considerably greater weight, but this cannot be regarded as true stimulation for the disease persisted on the surviving untreated plants and retarded their growth. The weight of treated plants is somewhat greater in the case of the smaller amounts of Semesan.

In experiments with Semesan applied at the rate of 0.5 gram to the square foot, good results have been secured on petunia, potato, zinnia, and aster seedlings. Some growers have been observed to use Semesan as early as one or two days after transplanting on certain seedlings. Some experiments with Semesan and copper sulphate used a full day after transplanting gave as good control as the immediate treatment but delaying until the second day resulted in poor control.

Drenching with Spergon

Spergon is the trade name for a recently introduced fungicide, tetrachloroparabenzoquinone. It is used to treat seed for the control of damping-off and has been found particularly effective in the case of peas. In the form intended for spraying or drenching, it contains a wetting agent and 50 per cent inert matter. That the wettable Spergon gives a high degree of control of damping-off when applied immediately after transplanting is shown by the experiments recorded in table 5 in which the material was used at the rate of 0.7 gram to the square foot.

The attack of Pythium was more severe than average but the degree of control was high and significant at 99 to 1 odds. The attack of Rhizoctonia was less severe than average and the stands of the treated flats were almost perfect. Control was significant at 99 to 1 odds. The seedlings exhibited neither growth stimulation

Table 5.—Control of damping-off in transplanted tomato seedlings by wettable Spergon applied immediately after transplanting at the rate of 0.7 gram to the square foot on artificially infested soil.

Infestation and Treatment	Stand of Surviving Plants in 100-plant Tests				
	Replications				
	1	2	3	4	Average
Pythium infested					
(a) Spergon treated	92	98	91	92	93.25
(b) Untreated	82	80	65	61	72
Rhizoctonia infested					
(a) Spergon treated	99	100	99	100	99.5
(b) Untreated	92	93	94	89	92

Fig. 2.—The comparative effects upon tomato growth and the control of damping-off from various soil treatments before transplanting, photographed after 3 weeks: **A.** Copper sulphate 0.9 gram to the square foot, 98 per cent stand; **B.** Steam treatment, 100 per cent stand; **C.** No treatment, 74 per cent stand.

Fig. 3.—Comparative effects of soil treatments continued: **D.** Copper sulphate 0.45 gram to the square foot, 98 per cent stand; **E.** Semesan 0.9 gram to the square foot, 99 per cent stand; **F.** No treatment, 94 per cent stand.

nor retardation. McNew *(9)* reports that tetrachloroparabenzoquinone causes a true stimulation in peas in addition to that which might be due to the control of fungi. The dosage used in the present tests on tomatoes is relatively strong and possibly too great to result in stimulation.

Typical examples of the effects of copper sulphate and Semesan soil treatments at transplanting in comparison with pasteurization by steam and no treatment are illustrated in figures 2 and 3. These results were obtained during fair weather in May but the transplanted seedlings were subjected to humid conditions using a muslin rather than a glass moist chamber. The stimulating effects of copper sulphate are observable in the greater height of the plants, A and D. In the case of Semesan, E, in which 0.9 gram was applied, growth appears slightly less advanced than in the cases of copper sulphate and steam pasteurization but the results are superior to no treatment.

OBSERVATIONAL DATA AND DISCUSSION

The comparison of tomato seedlings started in steamed and untreated soils and in two types of sand supplied with four different nutrient solutions gives no evidence that lack of balance or the omission of certain plant elements in sand culture are distinctive causes of severe damping-off which may follow transplanting to infested soil. While experimental results indicate minor differences in susceptibility probably due to nutrition, these differences do not appear primarily important. Properly washed sand supplied with superphosphate and saltpetre, which constitutes a practical preparation for growers, produces virtually as good results as steamed soil on the basis of the percentage of damping-off incident to transplanting.

Although the dilute unbalanced solution was found inferior to the superphosphate-saltpetre and Knop's, it was not markedly so and provided ample nutrition in some cases. This solution gave relatively greater losses in a few instances when the seedlings were transplanted to pasteurized soil, and this is probably an indication of physiological weakness. The concentrated 3-salt solution is in general equal to the superphosphate-saltpetre and Knop's but there is some evidence that it involves danger of chemical injury if the sand becomes very dry.

Improper Sand Culture Methods

Observation of results in growing tomato seedlings in sand by commercial growers during the present investigation has shown that important causes of trouble are (a) the use of natural sand without washing in hot water, (b) the growth of a second crop

without rewashing the sand, and (c) the application of unnecessarily large amounts of nutrient salts. Trouble has been characterized by chemical injury in conjunction with limited to moderate amounts of damping-off. It has usually followed a period of unseasonably hot, clear weather when greenhouse temperatures became unfavorably high, or around 100° F., and evaporation from the sand was rapid. Injury caused by heat and a too concentrated nutrient solution apparently predisposed the seedlings seriously to any damping-off organisms present. Both Pythium and Rhizoctonia may develop where the sand is not washed. Whereas sand tends to limit the spread of the fungi, their growth is favored by old dead seed and roots unless these are washed out and all contamination destroyed.

The accumulation of unused nutrient salts such as may arise when two or more crops are grown without washing may become great. In a bed of sand 5 inches deep, 20 grams (0.7 ounces) of soluble matter to the square foot was found after the nutrients for two crops had been applied by one grower. Of this total, 3 grams were present in the natural sand. The application for each crop had been two ounces of potassium nitrate and 1 ounce of calcium acid phosphate on 10 square feet, or 5.68 grams and 2.84 grams respectively of each salt per square foot. Apparently, very little of the nutrient salts were used or leached away, or matter dissolved in the water supply tended to increase the total. Considerable Pythium was present in this case. Although no trouble was noted in the first crop, it became serious in the second.

The likelihood of chemical injury by an excess of soluble matter depends much upon how low the moisture supply becomes as well as upon temperature. The nature and combination of the chemical ingredients is doubtless a factor, but this need not be considered here. An ounce of soluble matter per gallon of capillary moisture (7.5 grams per liter or 7500 parts per million) is an approximate danger point in the case of the two or three nutrient salts commonly used in sand culture. This point was closely approached in the experiments with excess potassium sulphate in the flats of quartz sand, which could retain two liters of water by capillarity. The superphosphate-saltpetre nutrients plus 9 grams of potassium sulphate made a total of 13 grams of soluble matter or 6500 parts per million. Injury was avoided by a high level of moisture. When the excess of potassium sulphate was increased to 12 grams, making 16 grams of soluble matter or 8000 parts per million, there was definite chemical injury and retarded growth.

In the bed of the grower previously mentioned, the specific gravity of the sand was 1.7 and its water-holding capacity was 18

per cent. This indicates that a square foot area 5 inches deep could retain 3.6 liters or .95 gallon. Twenty grams of soluble matter in this amount of moisture is 5580 parts per million. Full water holding capacity cannot be maintained, while somewhat less than half is best for plant growth. In this case, the point of injury was passed before the moisture fell to half of the capacity. Severe chemical injury occurred with a high rise of temperature and rapid drying. It is important to wash out soluble matter in natural sand since it presence leaves a more narrow margin of safety in case the moisture in the sand falls very low.

Favorable Conditions for Plant Growth

In transplanting healthy seedlings to naturally infested soil or to that infested with fresh oospores and conidia, losses were usually less than 15 per cent. There were exceptional cases with all sources of seedlings, however, in which losses by Pythium attained 50 per cent or more. The particular interaction of environmental factors producing such high incidence of infection is a matter of special interest. The manner in which environmental factors affect the seedlings, or host plants, as well as the parasite need to be considered.

A noteworthy observation in instances of excessive loss was that the period during which the seedlings were prone to wilt after transplanting was longer than ordinary. In some cases this was due apparently to exposure to sunlight, high temperature, and rapid transpiration but in other cases to low temperature and wet soil. In either instance, the conditions were extreme and did not favor the immediate renewal of growth, and pre-disposition to damping-off was increased accordingly. Hence optimum growing conditions tend to prevent damping-off.

Limitation of moisture in the seed flats does not induce any important degree of resistance, at least in seedlings transplanted a comparatively short time after emergence. Seedlings from steamed soil succumbed readily to infestation of the transplanting soil by actively growing mycelium although the seed flats were limited in moisture to the approach of plant wilting for several days prior to transplanting and the seedlings had attained an age of 16 days. Wide variation in the water content of the sand had no appreciable effect on later resistance. The addition of excess potassium sulphate gave a restricted yet significant decrease of damping-off, but in the experiments it was necessary to maintain a high level of moisture in the sand to prevent chemical injury.

Value of Disease-free Seedlings

In conformity with the report of Dunlap *(4)* untreated soil in the present investigation yielded fewer plants than sand culture

from a given amount of seed with approximately one-fifth of these unsuitable to transplant on account of disease or poor form. In this connection, it should be considered that the seed in both cases had been treated with a disinfectant. The greater yield of desirable seedlings seems sufficient reason in itself for maintaining disease-free seed beds.

The comparisons made in transplanting both healthy and infected seedlings into both infested and steamed soils provide a gage for the value of freedom from disease in seed beds. Transplanting from untreated soil to steamed soil did not prove a potent cause of loss, although the losses were relatively great in comparison with healthy seedlings. Seedlings from steamed soil and sand culture gave in certain tests reductions of approximately half in comparison with those from untreated soil in transplanting to infested soil, but this held true only under conditions favoring moderate disease. Study of the conditions in the seed flats and during transplanting indicates that environmental factors that favor the development of damping-off fungi in the transplanting soil far outweigh in importance the factors of resistance and source of seedlings. Severe losses may be sustained by healthy seedlings.

Control by Drying Transplanting Soil

When conditions are sufficiently warm and moist and appropriate organic matter is available, the development of Pythium is rapid, yet the fungus is very sensitive to drying as shown by the fast decline in potency of the inoculum on steamed rye. Experimentally, a high incidence of infection was assured only by infesting the surface half inch of soil not more than a day before transplanting. The activity of the parasite may rise quickly and subside in like manner. Apparently, the development of mycelium must immediately precede or coincide with transplanting to result in severe loss.

Keeping infested soil dry until the time it is used for transplanting is definitely an important deterrent to the later occurrence of damping-off. Loss may be limited by this precaution to a low percentage, a perfect stand not being impossible, even though conditions favorable to the disease may soon ensue. Seedlings acquire new roots and normal turgor before the parasite, which is present only in spore form, can develop mycelium for attack. The moistening of the soil to facilitate transplanting should be deferred until just before this operation.

Control by Fungicidal Drenches

Although drying infested soil and maintaining the most favorable conditions for plant growth can wholly avoid damping-off

after transplanting, circumstances may be such that the required conditions are not fully provided. Other effective control measures should be available. Fungicidal sprays and drenches after seedlings have put forth new roots and regained normal turgor have a degree of value, particularly against Rhizoctonia, but evidence is at hand showing that these measures may be too late in the case of Pythium, as nearly all loss occurs within three days. Data are reported here which demonstrate that safe and efficient control is possible by applying comparatively weak fungicidal drenches to prepared flats of soil just before or after transplanting in the case of tomato and a few other kinds of seedlings.

Copper sulphate at the rate of 0.5 to 1 gram to the square foot (0.5 to 1 ounce to 30 square feet) is effective against Pythium with the best results given under conditions of moderate attack. Semesan at the rate of 0.25 to 0.5 gram to the square foot (0.25 to 0.5 ounce to 30 square feet) is effective against both Pythium and Rhizoctonia. Wettable Spergon at the rate of 0.7 gram to the square foot (0.75 ounce to 30 square feet) is effective against both fungi. Water to dissolve or suspend these materials may be used at the rate of a pint to a square foot.

In applying these treatments to seedlings just after transplanting it is advisable to rinse the fungicide from the stems and leaves with a limited amount of pure water. The plants should be placed where the foliage will dry readily but not in direct sunlight. The moist chamber experiments indicated that some injury may occur under humid conditions. If such conditions prevail, treatment of the soil before transplanting may be undertaken.

SUMMARY

Experiments designed to determine whether lack of balance or the omission of certain plant elements in sand culture were causes of unusual susceptibility to damping-off in tomato seedlings gave no distinctive evidence of such relation. No significant difference was found between properly grown sand culture seedlings and those from steamed soil when transplanted to infested soil. There was evidence, however, that overconcentration of nutrient salts which results in chemical injury is a cause of susceptibility.

Washing the sand in hot water is essential for each seedling crop in order to remove excess soluble salts and organic matter and to destroy fungi. Considerable development of damping-off fungi was found to occur when such washing was omitted.

Reduction in the moisture content of seed flats did not affect appreciably the resistance of seedlings transplanted a comparatively short time after emergence. Extra potassium sulphate gave

a small but significant increase of resistance but a high level of moisture was required to avoid chemical injury.

After transplanting, seedlings were found highly susceptible to *Pythium ultimum* for about three days but to *Rhizoctonia solani* for a much longer or indefinite period.

Thoroughly drying Pythium infested soil very largely prevented damping-off of transplanted seedlings as they pass their highly susceptible period before this fungus can develop mycelium for attack. Environmental factors that favor prompt renewal of growth and a return of normal turgor reduced damping-off.

Freedom from disease on the part of seedlings from sand culture or steamed soil may reduce losses approximately half in comparison with those from untreated soil. But this holds true only under conditions of moderate disease. In averting severe losses, the prevention of conditions that favor the development of damping-off organisms in the transplanting soil is much more important than the resistance or source of the seedlings.

When other preventive measures are not undertaken, safe and effective control of damping-off in transplanted tomato seedlings is possible by applying comparatively weak drenches of copper sulphate, Semesan, or Spergon to prepared flats of soil just before or after transplanting. This early application is important as severe losses by Pythium occur during the first three days. This paper records experimental work in such use of fungicides.

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